EXHIBIT B

(10 Pages)

M-190

SEPHADEX-INDUCED INFLAMMATION IN RAT LUNG.
I. MODEL DESCRIPTION AND PROTECTIVE ACTION BY DRUGS.

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Inflammation and edema have pathophysiologic importance in several bronchial and alveolar diseases. Rather few small animal models have been described allowing the testing of anti-inflammatory action of intratracheally instilled (i.t.i) or inhaled drugs on these conditions. A new model has been developed, based on i.t.i. Sephadex beads (5 mg/kg). The beads provoke bronchial and pulmonary inflammation by a combination of allergic and immunologic mechanisms. Sephadex consists of dextran, to which rats have an endogenous hypersensitivity. Besides, the particular form probably triggers inflammation also via other immunologic mechanisms. Intrapulmonary pressure (measured according to Konzett-Rössler) and wet lung weight rise only slightly for the first hours, while there is a great rise during the interval 7-20 h. This rise is ascribed to the simultaneous development of a profound interstitial lung edema. As studied by bronchoalveolar lavage there is a marked infiltration into airways of neutrophils (from 3 h and onwards), of eosinophils (from 7 h) and later also of monocytes and lymphocytes.

Based on the gain of lung weight 20 h after Sephadex instillation, the anti-inflammatory activity of different types of drugs has been studied. Drugs known to reduce enhanced vascular permeability (e.g. the GCS budesonide and the β_2-stimulant terbutaline) can effectively block the edema. The NSAID indomethacin has no protective action. A high dose of diethylmaleate (a known depletor of glutathione) effectively inhibits the edema. The efficacy of GCS and of diethylmaleate, but the the lack of efficacy of indomethacin would together suggest that glutathione-containing leukotrienes are important inflammatory mediators. The edema can also be blocked by scavengers such as catalase, DMSO and DMTU, which suggests that hydrogen peroxide and some types of oxygen radicals contribute. However, SOD and iron chelators have no anti-edema efficacy.

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SEPHADEX-INDUCED INFLAMMATION IN RAT LUNG II. LIGHT AND ELECTRON MICROSCOPIC STUDIES.

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The major reason to that animal models of disease are developed is to reproduce human disorders in order to study the pathogenesis and pathophysiology of the process. The optimal hypersensitivity model would show similar picture of human disease and allow to study individual components of the hypersensitivity response. In abstract I a rat model of lung inflammation is described, where intratracheal instillation of Sephadex beads leads to an inflammation in the respiratory airways and alveolitis. The inflammation is probably induced partly by allergic mechanisms, as the Sephadex bead consists of dextran to which rats have an endogenous hypersensitivity. Pulmonary tissue specimens were examined by light and electron microscopy after 15 min., 2, 4, 7 and 24 hours and after two weeks of Sephadex or NaCl instillation. Microscopically an immediate response was seen at 15 min. as a widespread perivascular edema, probably due to increased venular permeability. Endothelial cells were swollen. Later a mixed cellular infiltration with a prominent eosinophilia was noted, mainly around the respiratory airways. Respiratory airways mucosa showed an asthma-like reaction with smooth muscle contraction, edema, infiltration of inflammatory cells with a striking increase in eosinophils. Sloughing of the epitelial lining cells and mucosa desquamation was observered. Mast cells in various stages of degranulation were seen between bronchial epithelial cells. Most of the Sephadex beads were spread rapidly in both alveolar and subpleural tissue. Around the beads there was a heavy granulomatous reaction, which could be noted in all specimens. Granulomas were mainly composed of monocytes and eosinophil cells with some giant cells of foreign body type. The inflammatory reaction was most prominent after 24 hours. All changes were resolved after two weeks. Owing to the particulate form of the Sephadex beads general immunologic mechanism may contribute to a granulomatous reaction. Sephadex induced reaction of the lung tissue is suitable as a model for study of: 1. Interstitial edema, 2. Asthma-like reaction on airway mucosa and 3. Granulomatous alveolitis.

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A rat model for testing anti-inflammatory action in lung and the effect of glucocorticosteroids (GCS) in this model

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It has been observed in patient studies that a better differentiation between antiasthmatic action in the lung and inhibition of adrenal function is reached by inhaled GCS (e.g. budesonide BUD) than that attained by oral GCS [1]. The reasons for the better differentiation reached by inhaled GCS are not known. One proposed but still unproved reason is that inhaled GCS act by local (topical) activity on airway mucosa and lung microvasculature, before they are absorbed and 'diluted' in the systemic circulation. The aim of the present study was to create a GCS sensitive airway inflammation in a rat model and to study the importance of the route of GCS administration for the

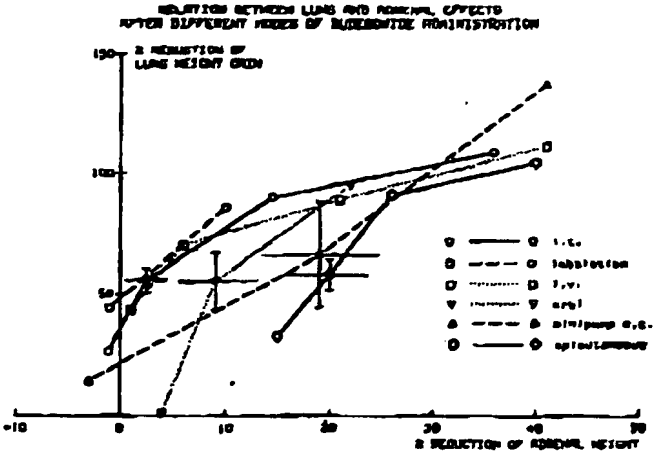

Figure 1
Relation between the reducing effects on lung edema formation and the adrenal weight, as influenced by the route of BUD administration. i.t. = intratracheal instillation, inhal. = inhalation, i.v. = intravenous injection, oral = oral administration, minipump s.c. = continuous release from s.c. implanted minipumps (Alzet®), epicutaneous = topically applied on a shaved area on the back skin. For doses inhibiting the lung edema formation by about 50% the s.e.m. are outlined. $N = > 6$ rats per dose level.

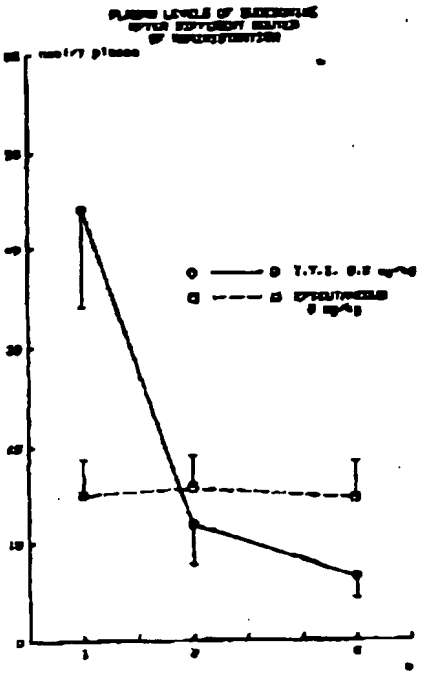

Figure 2
Plasma levels of BUD after i.t. instillation, 0.3 mg/kg, or epicutaneous application, 3 mg/kg. Mean ± s.e.m. of 5 rats.

relationship between anti-inflammatory action in lung and inhibition of hypothalamus-pituitary-adrenal axis.

Bronchiolitis and alveolitis were induced in male SD rats (220 g) by intratracheal (i.t.) instillation of Sephadex beads (5 mg/kg). Sephadex consists of dextran to which rats have an endogenous hypersensitivity [7]. There is no immediate reaction, but after some hours the beads attract neutrophils, eosinophils and macrophages. These form cuffs around the beads, which are situated for the first hours in bronchioles and later on also more peripherally. The histological picture is a focal bronchiolitis and alveolitis leading to a peribronchial and interstitial edema and to impaired ventilation. These pathological changes can be quantified by the gain of lung wet weight, the rise in which correlates in time with the infiltration of granulocytes (esp. of eosinophils) and with the impaired ventilation. After one day the lung weight increases by 50–75%, the weight gain persisting for at least 4 days.

BUD given by i.t. instillation 30 min before Sephadex counteracted partly (dose 0.1 mg/kg) or nearly completely (dose 1 mg/kg) the wet lung weight gain, impaired respiration, histological changes and influx of eosinophils [3]. To study if the protection induced by i.t. instillation depends on a local action, a refined model was used in which only the left lung lobe was pretreated with instilled BUD while Sephadex was given to both lung halves. The consistent result of such tests has been that the same protection against edema was obtained in the right as in the locally treated left lung lobe. This demonstrates that the anti-edema efficacy of i.t. instilled BUD does not rest on a local action at the application site in lung.

To be able to study adverse effects on adrenal function Sephadex-treated rats were given BUD twice a day for four days. At sacrifice the gain in wet lung weight and the adrenal weight were determined. Six routes of BUD administration were tested and the results are given in Fig. 1. When BUD was given by i.t. instillation, inhalation or i.v. injection, it was possible to inhibit the lung edema formation by up to 50% without reducing the adrenal weight. Such a differentiation between lung and adrenal effects was not attained with three other routes tested (Fig. 1). Diminished lung edema formation by 50% was then coupled to reduction of the adrenal weight by ~10% (after oral administration) or by ~20% (after epicutaneous or continuous from s.c. minipumps). Thus, the route of BUD application affected the relationship between lung and adrenal effects markedly but there was no simple correlation between selective lung action and the local mode of application to the lung.

The levels of circulating BUD in plasma were determined after 5 routes of administration: i.t. and i.v. representing routes differentiating between lung and adrenal effects and epicutaneous as a route without such separation. Doses with about the same anti-edema efficacy in lung were selected (i.t. and i.v. 0.3–0.4 mg/kg and epicutaneously a dose about ten times higher). BUD was determined with a RIA method [4]. I.v. and i.t. administration gave rather similar plasma levels from 5 minutes onwards, but the i.v. values were higher for the first 2 minutes. After i.t. instillation the plasma levels of BUD were ~2000 after 1 minute, after 1 h ~40, after 3 h ~10 and after 6 h ~6 nmol/l. Epicutaneous administration gave no clear plasma peak, and the levels were stable at ~15 nmol/l between 1 and 6 hours. Figure 2 shows one experiment comparing the BUD levels in plasma after i.t. and epicutaneous application. At 1 h the epicutaneous levels were 3 times lower than the i.t. ones ($p < 0.05$), while at 6 h the opposite relation between the two application routes was noted.

The profile demonstrated by i.t. instilled or by inhaled BUD, marked anti-edema action in lung but low activity on adrenal weight, supports the relevance of the rat model, as inhaled BUD gives a principally similar differentiation in asthmatic patients. As studied in the rat model, the following proposals can be raised currently as reasons for lung selectivity reached by i.t. instillation:

—it seems not to depend on a local action of BUD at the application site in lung;

—analyses of BUD in plasma show that i.t. instillation leads to a very rapid systemic absorption of BUD. The plasma peak following i.t. administration is seen at ~1 minute, after which time the plasma levels drop rapidly. Epicutaneous administration leads to lower but more protracted BUD levels. It is suggested that these different types of plasma curves can explain why the former but not the latter administration route differentiates between lung and adrenal activity. If so, a rather short plasma peak of BUD is

sufficient to trigger an anti-edema effect in the lung, possibly by induction of proteins with anti-inflammatory action [5]. Recent studies in our lab in the hamster cheek pouch model show that as large anti-edema efficacy can be reached by BUD given i.v. as by local application of BUD to the cheek pouch for 5 to 60 minutes [6]. The effectiveness of local application for only 5 min may suggest that BUD exerts its effect locally via endothelial cells in the exposed microvasculature. For induction of the principles inhibiting the hypothalamus-pituitary-adrenal axis, the duration of circulating BUD may be more important than the height of the peak.

An alternative explanation for the differentiation may be that the distribution of BUD between lung and brain tissue (the adrenal involution starts probably at the brain level) varies with the height of plasma levels, so that high and short peak levels would favour binding to lung tissue while prolonged plasma levels may lead to relative enrichment in brain.

Further experimental investigations are required to critically evaluate these two hypotheses.

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Original Paper

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Intratracheal Application of Sephadex in Rats Leads to Massive Pulmonary Eosinophilia without Bronchial Hyperreactivity to Acetylcholine

Key Words
Bronchial hyperreactivity
Pulmonary eosinophilia
Asthma
Sephadex
Animal model

Abstract
Fourteen Brown-Norway rats were pretreated with physiological saline (n = 7) or 500 µg Sephadex (n = 7) intratracheally. 24 h later, a bronchial provocation test was performed under pentobarbital anaesthesia using increasing doses of acetylcholine aerosol and the degree of bronchospasm was measured using a modified Konzett-Rössler method. Subsequently, leucocyte counts were determined in the bronchoalveolar lavage fluid (BALF), BALF cells were differentiated, and the chemiluminescence of the BALF leucocytes were measured. Finally, the lungs were removed and histologically examined. The cell count in the BALF was significantly ($p < 0.05$) increased in the animals pretreated with Sephadex compared to those in the saline group (mean value ± SEM: 0.38 ± 0.07 vs. 0.15 ± 0.02 × 10^6/ml). This difference was also reflected in the chemiluminescence measurements (2.51 ± 0.53 vs. 0.20 ± 0.03 × 10^6 counts/0.5 ml). In the Sephadex-treated animals there was also a significant increase in the absolute number of neutrophil (0.040 ± 0.010 vs. 0.011 ± 0.002 × 10^6/ml) and, in particular, eosinophil granulocytes (0.188 ± 0.055 vs. 0.003 ± 0.001 × 10^6/ml) in the total leucocytes of the BALF. Lung histology showed massive perialveolar and peribronchial oedema and granulomatous infiltrates, primarily with eosinophils, after intratracheal application of Sephadex; these findings were not observed in the saline group. None of these changes in the rats pretreated with Sephadex manifested themselves in increased bronchial reactivity to acetylcholine aerosol. It is uncertain if the Sephadex-induced increase in the eosinophil count is accompanied by an activation of this cell population, which appears to be of importance for the occurrence of bronchial hyperreactivity. It is clear from this animal model that the presence of inflammatory cells in the BALF and lung tissue of rats does not in itself result in increased reactivity of the airways.

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Introduction

Bronchial asthma is a chronic disease whose primary symptoms are reversible bronchospasms and inflammation of the airways with increased reactivity to a large number of irritants. Biopsies of the bronchial mucosa of asthmatics show characteristic histological changes: infiltration with leucocytes, in particular, with eosinophil granulocytes, oedema, epithelial desquamation, hyperplasia of the mucous glands and hypertrophy of smooth muscle [1]. These changes are also found in patients with mild, stable asthma, and a correlation between the degree of inflammation and the degree of bronchial hyperreactivity has been reported [2]. There has recently been increased discussion of whether eosinophils play a central role in the occurrence of airways hyperreactivity [3–5]. Damage of the bronchial epithelium by toxic, basic eosinophilic proteins [6] with resultant suspension in the production of a factor which relaxes the airway smooth muscle [7] has been cited as a possible pathogenetic factor. Furthermore, afferent nerve endings are exposed as a result of epithelial damage. Stimulation of these nerve endings may then lead to the release of bronchoconstricting and pro-inflammatory neuropeptides via an axon reflex [8]. As well as toxic proteins, eosinophils can also release other pro-inflammatory mediators such as leukotrienes [9] and reactive oxygen species [10]. Because of the possible pathogenetic significance of pulmonary eosinophilia in asthma, it is important that this effect is also found in an animal model for this disease. Walls and Beeson [11] were able to produce blood eosinophilia in rats by intravenous (i.v.) injection of Sephadex (cross-linked dextran) particles. In addition, there were increased numbers of eosinophils in the airways accompanied by hyperreactivity to i.v. 5-hydroxytryptamine [12, 13] and aerosols of 5-hydroxytryptamine and ovalbumin in sensitised animals [14]. Despite embolisation of the pulmonary vessels, it was not possible to reproduce this effect by i.v. injection of latex particles [15], which points to a Sephadex-specific effect for the eosinophilia, possibly as a result of the known endogenous dextran hypersensitivity of rats [16]. Inbred Brown-Norway rats appear to be a suitable rat strain for an animal model in asthma, since this strain is characterised by a high immunoglobulin E response to sensitisation to antigen and by blood eosinophilia [17, 18]. For this reason, we decided to test intratracheal application of Sephadex in Brown-Norway rats in order to determine whether a non-specific bronchial hyperreactivity to acetylcholine aerosol resulted from the expected eosinophilic inflammation.

Methods

Details of the methods have already been reported by us elsewhere [14, 19]. The most important technical details are summarised below.

Animals and Pretreatment

Male Brown-Norway rats (Charles River Wiga, USA) aged 12–14 weeks and weighing 250–300 g were non-operatively intratracheally instilled with either 100 µl physiological saline or 500 µg Sephadex-G 200 (Pharmacia, Uppsala, Sweden) in 100 µl saline via a plastic tube under short-term inhalation anaesthesia with methoxyflurane (Penthrane®, Abbott, Chicago, USA; 7 animals per group). The Sephadex suspensions used were prepared under sterile conditions 2 days before the investigations and stored at 4 °C.

Measurement of Bronchoconstriction

Twenty-four hours after pretreatment, the animals were anaesthetised with 60 mg/kg pentobarbital (Narcoren®, Rhone Merieux GmbH, Laupheim, FRG) intraperitoneally and their tracheas cannulated and connected to a ventilation pump (60 strokes/min, 2–4 ml). The strength of the bronchospasm to aerosol applied via the ventilation pump (Rhema, Hofheim/Taunus, FRG) was measured as the increase in the respiratory resistance by means of an overflow-measuring unit at a constant pressure of 7 cm water column according to the method of Konzett and Rössler [20] as modified by Collier et al. [21] and Collier and James [22]. Increasing doses of acetylcholine aerosols, generated from stock solutions of 0.3, 1, 3, and 10 mg/ml saline, were each applied for 3 min using an ultrasonic nebuliser (Heyer USE 77, nebuliser power 4 ml/min, ventilator power 15 l/min, aerosol particle size 0.5–4 µm; Heyer, Bad Ems, FRG). The bronchoconstriction was measured for 10 min before application of the next higher dose. A direct effect of acetylcholine on the cell count in the bronchial lumen was ruled out in a pilot investigation in which non-pretreated animals ($n = 4$) were exposed to acetylcholine aerosol in the manner described above. Bronchoalveolar lavage (BAL) with subsequent cell counting and measurement of the chemiluminescence were then performed as laid out below. Compared with the values for a non-pretreated control group ($n = 4$) which underwent BAL without previous bronchoprovocation with acetylcholine, there was no significant difference between the parameters [data not shown].

Bronchoalveolar Lavage

Directly after measurement of bronchoconstriction, the cannulated trachea was connected to a perfusor pump (Braun, Melsungen, FRG) instilling 2 ml of 1 *mM* EDTA in saline. This solution was instilled into the bronchial system 3 times for 1.5 min and then re-aspirated before the lavage fluid (BALF) was brought on ice (yield: at least 80% of the instilled volume). This procedure was repeated twice more, using 2 ml saline/EDTA solution each time.

Pathohistological Investigation

Directly after lavage, the animals lungs (including the trachea) were removed and fixed in 10% formalin. Discs of tissue about 3 mm thick were taken from each lobe and embedded at 65 °C in Paraplast® (Sherwood Medical Industries, St Louis, USA) via an increasing alcohol series using xylene as intermedium. 4–5 µm sections were prepared from the embedded tissue discs, applied to uncoated slides, stained with haematoxylin-eosin, PAS and Giemsa and then examined by light microscopy.

Table 1. Cell count and chemiluminescence in the BALF of Brown-Norway rats

Pretreatment	Cell count ml × 10^6	Chemiluminescence counts/min/0.5 ml × 10^6
Saline	0.15 ± 0.02	0.20 ± 0.03
Sephadex	0.38 ± 0.07	7.51 ± 0.53

Intratracheal pretreatment with saline (n = 7) or 500 µg Sephadex (n = 7) 24 h previously. Values as mean ± SEM.

Table 2. Absolute numbers (ml × 10^6) and percentage populations of various cell types in the total cell count of the BALF of Brown-Norway rats depending on intratracheal pretreatment (saline, n = 7, or 500 µg Sephadex, n = 7, 24 h previously; values as mean ± SEM)

Pretreatment	Mononuclear cells	Eosinophils	Neutrophils
Saline	0.136 ± 0.027 (91%)	0.003 ± 0.001 (2%)	0.011 ± 0.002 (7%)
Sephadex	0.154 ± 0.011 (41%)	0.188 ± 0.055 (49%)	0.040 ± 0.010 (10%)

Cell Count and Cell Differentiation

Immediately after lavage, 1 ml BALF was mixed with 9 ml isotonic diluent (Nova Celltrak, Waltham, USA) and 3 drops of haemolytic reagent (Nova Celltrak) to lyse the few erythrocytes. The cell count was performed using a Coulter Counter ZM (Coulter Electronics, Luton, UK). The differential count of the leucocytes was performed by light microscopy following centrifugation (700 rpm, Labofuge A, Heraeus, Hanau, FRG) of the BALF on a slide with subsequent staining with a modified May-Grünwald stain (Diff-Quik®, Baxter Dade AG, Düdingen, Switzerland). 400 cells were counted from each preparation.

Determination of Chemiluminescence

200 µl BALF were mixed with 100 µl BM-86 Wieler buffer (Boehringer Mannheim GmbH, Mannheim, FRG) and 100 µl Luminol 2×10^{-4} *M* (Boehringer Mannheim GmbH), and incubated for 15 min at 37 °C. 100 µl opsonised zymosan (15 mg/ml) were then added and measurement was performed immediately with an autoluminat LB 950 (Berthold, Wildbad, FRG) for 15 min. Opsonisation was carried out by washing 50 mg zymosan (Sigma, St. Louis, USA) suspended in 1 ml saline twice with saline and then incubating the suspension with 1 ml of a pooled rat serum for 30 min at 37 °C.

Statistics

The distribution-free Wilcoxon test (U test) for non-normal populations was used to calculate the significance of a difference between two parameters. The level of significance was set at $p < 0.05$. The correlation coefficient r was calculated to determine the degree of linear dependency of two parameters. The significance of a correlation was calculated according to Fisher's method.

Results

Table 1 shows mean values ± the standard error of the mean (SEM) for the leucocyte count and chemiluminescence of the leucocytes in the BALF for the Sephadex and saline groups. The bronchopulmonary inflammation induced by Sephadex is reflected in the more than 2-fold increase in the leucocyte count and the 25-fold increase in chemiluminescence compared to the values for the saline group. The greater degree of interindividual scatter after pretreatment with Sephadex compared to saline is clearly evident. In all cases, there was a significant difference in the parameters in the two groups at $p < 0.05$. In the Sephadex animals there was a highly significant correlation between cell count and chemiluminescence ($r = 0.95$; $p < 0.01$) which was not observed in the saline animals ($r = 0.65$, not significant). Table 2 shows the absolute numbers and the percentage of mononuclear cells (mononuclear phagocytes and lymphocytes), neutrophil and eosinophil granulocytes in the BALF cells. Intratracheal administration of Sephadex resulted in an increase in both the percentage and absolute number of eosinophils in particular and, to a lesser extent, of neutrophils. By contrast, the percentage of mononuclear cells in the BALF fell, although the absolute values also increased to a certain degree. The difference in the absolute numbers of the individual cell types between the Sephadex and the saline animals was significant for eosinophils and neutrophils, but not for the mononuclear cells.

Histological investigation of the lung preparations from Sephadex-pretreated rats revealed pronounced peribronchial and perivascular oedema with heavy accumulation of eosinophil granulocytes and few mixed cell infiltrates (fig. 1a, d). PAS-positive Sephadex particles were widely distributed in the alveolar lumina. There was a clear granulomatous reaction around the Sephadex particles (fig. 1b). The disseminated granulomas localised in the lung tissue were composed of mononuclear cells and eosinophils. In some of the granulomas, the eosinophils were arranged concentrically around the alveolar lumina. The mononuclear cells were predominantly macrophages with reniform nuclei, sparse heterochromatin and vacuolar cytoplasma. Lymphocytes were found only rarely in the granulomas. Only isolated giant cells (fig. 1c), neutrophils and mast cells were observed. The rats which had re-

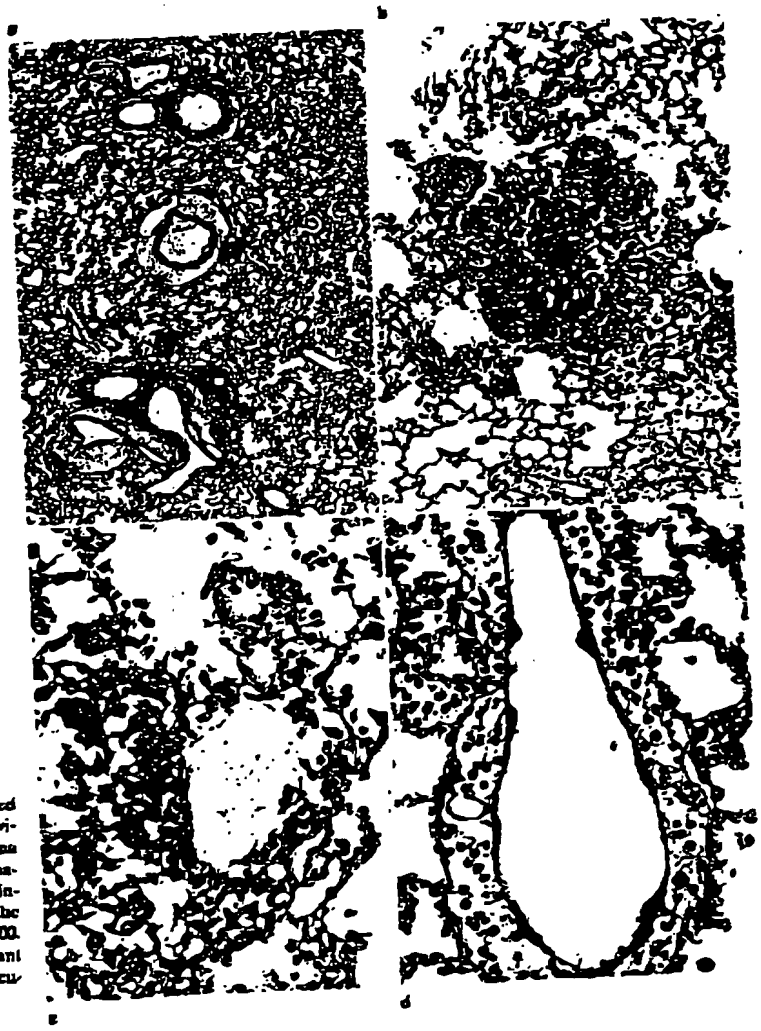

Fig. 1. Lung specimens of rats pretreated intratracheally with Sephadex 24 h previously. a Peribronchial/perivascular oedema and disseminated granulomatous inflammation 1:25. b Prominent granulomatous inflammation in the alveolar tissue around the positive PAS-stained Sephadex beads 1:100. c Granulomatous inflammation with a giant cell. 1:200. d Perivascular oedema with accumulation of eosinophils. 1:200.

ceived saline intratracheally exhibited only minimal peribronchial and perivascular oedema. Only isolated eosinophils and mononuclear cells were found in the pulmonary tissue. In spite of the massive inflammatory changes, hyperreactivity to increasing doses of acetylcholine aerosol (0.3, 1, 3, 10 mg/ml saline) was not found in Brown-Norway rats 24 h after intratracheal instillation of 500 µg Sephadex compared to the saline control group. The dose-response curves of bronchoconstriction for the two groups were virtually identical (fig. 2).

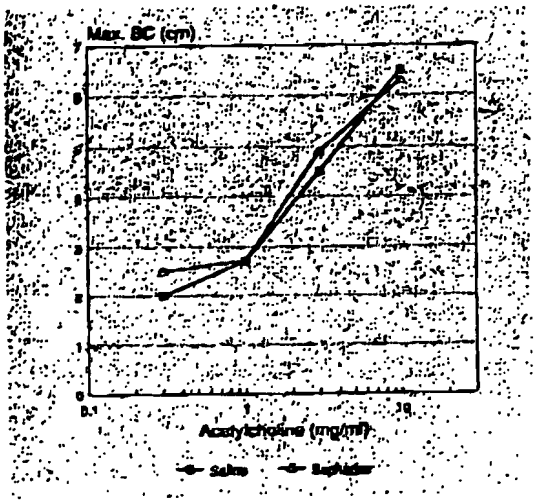

Fig. 2. Dose-response curves of maximal bronchoconstriction (max. BC) after administration of increasing doses of acetylcholine aerosol. No significant differences at any dose between Brown-Norway rats pretreated intratracheally with saline (■, n = 7) or Sephadex (Δ, n = 7). Values as mean.

Discussion

In this study we were able to demonstrate that despite massive eosinophilia of the airways and peribronchial tissue as a result of intratracheal application of Sephadex, bronchial hyperreactivity to acetylcholine aerosols was not found in Brown-Norway rats. Our results are in accordance with those of other authors, although they were obtained with different animal species and models. Sanjar et al. [23] also found no evidence of a correlation between airway eosinophilia and the degree of bronchoconstriction to ovalbumin aerosol in sensitised guinea pigs. Pretreatment with aminophylline, ketotifen or dexamethasone prevented eosinophilia but not hyperreactivity. Following subcutaneous or intraperitoneal administration of recombinant human granulocyte-macrophage colony-stimulating factor or interleukin-3 in guinea pigs, Kings et al. [24] reported eosinophilia in the BALF but found no signs of increased bronchial reactivity to i.v. histamine. Ishida et al. [25] showed that antigen-sensitised guinea pigs which were treated with an antagonist of platelet-activating factor (PAF) before multiple antigen provocation did not exhibit the airway hyperreactivity to acetylcholine aerosol shown by control animals which had received the antigen without pretreatment with the PAF antagonist. However, both groups of animals exhibited eosinophilia of the bronchial system. There are some possible explanations for the lack of a relation between airway eosinophilia and hyperreactivity in our and these animal models.

Recent experimental results increasingly suggest that it is not simply the presence of eosinophils but above all their degree of activation which is of importance for their ability to damage tissue. According to current understanding, interleukin-5, a product of activated T lymphocytes, plays a key role in the activation of eosinophils [26]. A positive correlation between the numbers of activated T cells, eosinophils and the amount of mRNA for interleukin-5 was observed in the bronchial mucosa of atopic asthmatics [27]. The number of activated eosinophils was reported to correlate with the degree of airway reactivity [28]. Furthermore, significantly elevated numbers of hypodense, activated eosinophils have been detected in the blood of asthmatics during a late-phase reaction [29]. All these observations underline the importance of activated eosinophils in the pathogenesis of chronic asthma.

Although in our animal model there was distinct eosinophilia in both the bronchial lumen and in lung tissue, little can be said about the degree of activation of these cells in the context of the investigations performed. After pretreatment with Sephadex as well as with saline only isolated lymphocytes were detected in the pulmonary tissue. In hyperreactive asthmatics clearly elevated numbers of mucosal T cells could be demonstrated [28], which might be important for the activation of eosinophils. The determination of chemiluminescence in the BALF primarily measures the phagocytosis activity of neutrophils and eosinophils. This increased by more than a factor of twenty 24 h after intratracheal application of Sephadex, correlating with the greatly elevated number of neutrophils and, in particular, eosinophils, and thus giving no indication whether eosinophil activation has taken place or not.

Although there was a significant increase in the absolute number of neutrophils in the BALF 24 h after intratracheal instillation of Sephadex, lung histology revealed only isolated neutrophils. This is probably due to the known kinetics of this cell type in response to an inflammatory stimulus: the maximum accumulation of neutrophils at the site of damage is achieved after only a few hours and then declines. The neutrophilia in the BALF is simply a reflection of the state prevailing in lung tissue a few hours earlier. The absence of distinct neutrophilia in lung tissue 24 h after intratracheal application of Sephadex in conjunction with the absence of simultaneous bron-

thial hyperreactivity could be possible evidence of the importance of this cell population for the occurrence of this symptom in the present animal model. The pathogenetic relevance in man is still discussed controversially, but distinct correlations between pulmonary neutrophilia and airway hyperreactivity have been reported in various animal models [1]. In this context it must be stressed that the histology of intratracheal Sephadex administration after 24 h only reflects certain parameters of human asthma. Asthma in man is a chronic process, often of allergic origin, while this animal model is only a short-term test with foreign body irritation of the bronchial tissue. In contrast to human asthma, granulomatous structures were observed in lung tissue in this model. The perivascular and peribronchial oedemata and the massive tissue eosinophilia, however, correspond well with the situation in asthmatics.

Recently, there have been increasing reports of a dissociation between inflammation and reactivity of the airways in man. Hargreave et al. [30] reported a connection between short-term increases in bronchial reactivity and pulmonary inflammation with mediator release, but this seems not to be definite in the case of chronic hyperreactivity in advanced asthma stages. In a noteworthy long-term study, Lundgren et al. [31] were able to show by means of biopsies of the bronchial mucosa and bronchial provocation tests that asthmatics who had been treated for 10 years with glucocorticosteroid aerosols no longer exhibited any signs of inflammatory infiltration in bronchial tissue although they were still hyperreactive to inhaled methacholine.

In summary, we were able to show in Brown-Norway rats that despite massive eosinophilia of the airways and lung tissue 24 h after intratracheal instillation of Sephadex, there was no increase in bronchial hyperreactivity to acetylcholine aerosol. Although the degree of activation of these cells is subject to speculation, it may be stated that in this animal model the absolute number of pulmonary eosinophil granulocytes does not permit positive conclusions on airway reactivity to be drawn. This is an observation which can probably also have to be applied to other animal models of asthma.

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